The complete amino acid sequence of the large bacteriochlorophyll-binding polypeptide B870α from the light-harvesting complex B870 of *Rhodopseudomonas capsulata*

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Received 19 July 1985

The larger (α) of the two bacteriochlorophyll-binding polypeptides of the antenna complex B870 of the photosynthetic apparatus of *Rhodopseudomonas capsulata* was extracted by chloroform-methanol-ammonium acetate from the intracytoplasmic membrane and purified by column chromatography on LH60. The complete amino acid sequence has been determined. The M_r of the polypeptide is 6588. The protein consists of 58 amino acids, having a polarity of 28%.

Bacteriochlorophyll-binding polypeptide *Primary structure* Rhodopseudomonas capsulata *B870 antenna pigment complex*

1. INTRODUCTION

The photosynthetic apparatus of the anoxygenic facultative phototropic bacterium *Rhodopseudomonas capsulata* contains the 2 light-harvesting complexes B870 and B800-850 designated by their in vivo near-infrared absorption bands [1,2]. The B870 antenna complex is synthesized together and associated with the photochemical reaction center; the B800-850 complex is synthesized independently and in variable amounts and surrounds the reaction center-B870 aggregates in the membrane [2]. Bacteriochlorophyll (Bchl) *a* and carotenoids are non-covalently bound to low-M_r polypeptides in both antenna complexes. Due to Bchl-protein and possibly Bchl-Bchl interactions, the long-wavelength absorption band of Bchl at 770 nm (dissolved in acetone-methanol) is shifted to 870 nm and 800 and 850 nm, respectively, in vivo when associated to membrane proteins. Both antenna complexes contain 2 pigment-binding polypeptides [1–4]. The amino acid sequence of the small Bchl-binding polypeptide (B870β, M_r 5341) has been determined [5]. Here we describe the complete sequence of the large pigment-binding polypeptide B870α.

2. MATERIALS AND METHODS

2.1. *Growth of bacteria and isolation of membranes*

Intracytoplasmic membranes were isolated from anaerobically light-grown cells of the strain A1a$^+$ of *Rps. capsulata* (crt$^-$, pho$^+$, B800-850$^-$) as described [6]. The polypeptides of the purified membrane and the isolated polypeptides were characterized by the Laemmli SDS-polyacrylamide gel electrophoresis procedure [1].

2.2. *Isolation of the polypeptide*

The polypeptides of the B870 complex were extracted from 80 mg freeze-dried membrane or 8 mg freeze-dried complex B870 with chloroform/methanol (1:1, v/v) mixture containing 0.1 M ammonium acetate and separated by chromatography on Sephadex LH60 (Pharmacia)

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00145793/85/$3.30

as in [5]. Peak fraction II (fig.1 in [5]) was isolated and the volume reduced from 100 to 3 ml by rotary evaporation. After addition of 6 ml chloroform/methanol (1:2, v/v) the extract was centrifuged (20 min, 15000 × *g*). The pellet was reextracted with the same solvent and again centrifuged. The supernatants were combined and rechromatographed on LH60.

2.3. *o-Iodosobenzoic acid cleavage and fractionation of the fragments*

Approx. 6 mg (0.8 μmol) protein was treated with 4 mg *o*-iodosobenzoic acid in 2 ml acetic acid (80%, v/v), 4 M guanidinium HCl according to [6]. After cleavage the mixture was applied to a column (1.8 × 95 cm) of Bio-Gel P6 (200–400 mesh), which was equilibrated and eluted with acetic acid (80%).

2.4. *Partial acid hydrolysis and fractionation of the fragments*

15 mg protein were incubated in 4 ml formic acid (75%, v/v) for 48 h at 40°C. The reaction mixture was fractionated on a BioGel P10 column (200–400 mesh, 2.2 × 90 cm). The column was equilibrated and eluted with formic acid (50%).

2.5. *Amino acid analysis*

Amino acid analysis was carried out with a Biotronic LC 6000 E amino acid analyzer. Samples were hydrolyzed at 106°C in 200 μl of twice distilled 5.6 M HCl for 24, 48 and 144 h in sealed and evacuated tubes.

2.6. *C-terminal amino acid sequence determination*

The sample was treated with carboxypeptidase Y at pH 5.5 as described [7].

2.7. *N-terminal amino acid sequence determination*

Automated N-terminal amino acid sequence was performed by Edman degradation with an automatic sequencer (Beckman 890C) using the quadrol (0.25 M) program together with polyprene as additive [8]. Conversion of the thiazolinones to the thiohydantoin derivatives of amino acids and the identification by HPLC were carried out as in [9]. PTH-arginine and PTH-histidine were separated by reverse-phase HPLC on a Nucleosil C8 column using the isocratic solvent system 25 mM sodium acetate/acetic acid, pH 6.5, 19% methanol.

3. RESULTS

3.1. *Isolation of the polypeptide B870α*

The polypeptides of the B870 light-harvesting pigment-protein complex were extracted and purified as described in section 2 and in [5]. The peak fraction II (fig.1 in [5]) yielded after rechromatography the polypeptide B870α (terminology, see [10]). It ran to the same position as the large polypeptide of the B870 complex extracted from the membrane (fig.1) or from the isolated complex [3]. Only one band was obtained in polyacrylamide gel electrophoresis. By Edman degradation only one N-terminal sequence was obtained.

3.2. *Sequence determination*

The N-terminus of the purified polypeptide was found to be blocked in Edman degradation. After

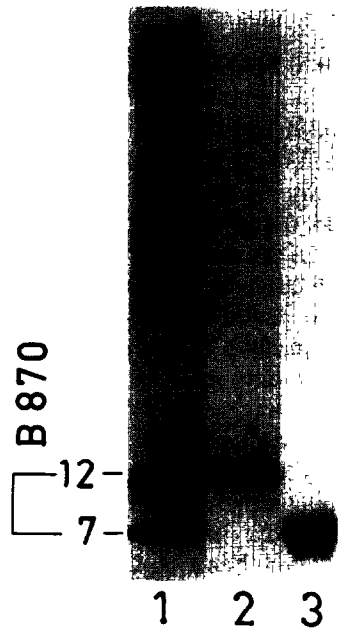

Fig.1. SDS gel electrophoresis of polypeptides (11.5–16.5% acrylamide gradient). Lanes: 1, membrane of *Rps. capsulata*, strain A1a$^+$; 2, purified polypeptide B870α; 3, polypeptide B870β. 12 and 7 indicate the apparent M_r determined by PAGE.

treatment of the polypeptide with 5% (v/v) concentrated HCl in chloroform:methanol (1:1, v/v) for 24 h at room temperature [11] 34 N-terminal amino acid residues could be determined (fig.2).

3.3. *Partial acid hydrolysis of the polypeptide B870α*

After partial acid hydrolysis (see section 2) the reaction product was applied to BioGel P10 chromatography. Elution patterns (fig.3) and amino acid determination (not shown) indicated that fraction P1 consisted mainly of uncleaved polypeptide, fraction P2 contained residues 13–58 and fraction P3 the N-terminal peptide with amino acids 1–12. Automated Edman degradation of the P2 fraction resulted in the sequence of 34 amino acid residues from position Pro 13 to Val 46 (fig.2).

3.4. *Peptides from o-iodosobenzoic acid cleavage*

6 mg polypeptide B870α was treated with *o*-iodosobenzoic acid as described in section 2. The cleavage products were separated on BioGel P6. Amino acid analysis indicated that peak fraction 0–1 (fig.4) contained mainly residues 9–43. Fraction 0–2 (fig.4) contained a peptide with the sequence Leu-Thr-Val-Ala-Thr-Ala-Lys-His-Gly-Xaa-Val-Ala-Ala-Ala-Gln-COOH. Thus this fragment comprises the segment following position 43 (Trp, fig.2). Position 53 could not be identified by sequence analysis. Amino acid analyses of fraction 0–2 and of the complete polypeptide suggested that tyrosine had been degraded by *o*-iodosobenzoic acid/guanidinium chloride treatment. *o*-Iodosobenzoic acid cleavage was repeated as described in section 2 but in the presence of 15.6 mg free tyrosine to protect the tyrosine at position 53. After chromatography (not shown) only a single peak corresponding to the uncleaved polypeptide was obtained. Most probably the addition of free tyrosine to the reaction mixture inhibited the cleavage reaction. The presence of 2 tyrosine residues in the amino acid analysis of the

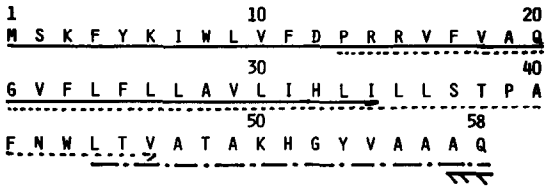

Fig.2. Complete amino acid sequence of the large bacteriochlorophyll-binding polypeptide of the B870 complex (B870α); ——→, N-terminal sequence of B870α in the sequencer; – – – –→, sequence determination of the C-terminal fragment obtained after partial acid hydrolysis; ·–·–·–→, sequence determination of *o*-iodosobenzoic acid C-terminal fragment; ⟵, C-terminal amino acid sequence determination.

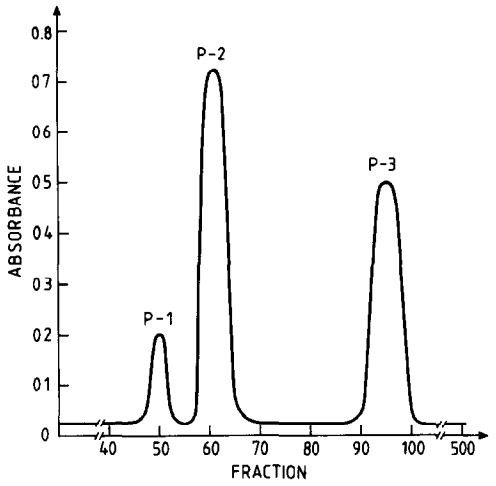

Fig.3. Gel filtration of the partially hydrolyzed B870α polypeptide on BioGel P10 (200–400 mesh). Flow rate 1.8 ml/h. 500 fractions were collected, each containing 1.8 ml.

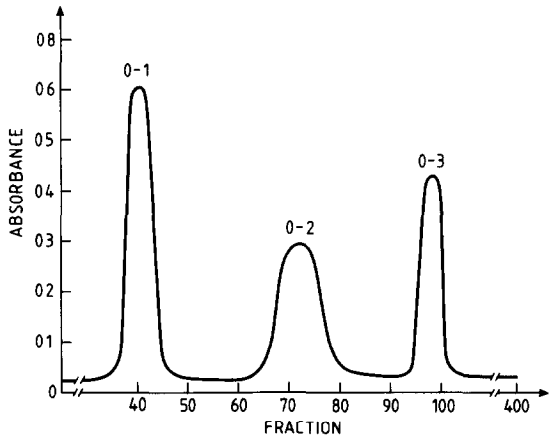

Fig.4. Gel filtration of *o*-iodosobenzoic acid-cleaved B870α polypeptide on BioGel P6 (200–400 mesh). Flow rate 2 ml/h. 400 fractions were collected with 2 ml each.

total protein and the deduction of the tyrosine in position 53 from the nucleotide sequence determination [12] gave strong evidence that tyrosine is in position 53.

C-terminal analysis of fragment 0–2 and of the whole polypeptide by carboxypeptidase Y treatment resulted in the same degradation patterns revealing the C-terminal sequence Ala-Gln. Glutamine as the C-terminal amino acid has been confirmed by N-terminal sequencing of the 0–2 fragment, amino acid analysis of the whole polypeptide and of the C-terminal peptide 0–2 and deduction from the nucleotide sequence [12].

4. DISCUSSION

The large Bchl-binding polypeptide B870α has an apparent M_r of 12000 shown by SDS-polyacrylamide gel electrophoresis [3]. On the basis of amino acid sequence analysis the polypeptide consists of 58 amino acid residues having an M_r of 6588. The polarity determined by the criteria of [13] was 27.6. The polypeptide contains a hydrophilic N-terminal region (positions 1–16). The remaining polypeptide including the C-terminal region is hydrophobic. Thus the remarkable difference between the apparent and actual M_r values may be explained as an effect of a structure which differs strongly from the structure of water soluble proteins which are normally used as standards for PAGE.

The N-terminal regions of B870α and B870β, which are believed to be exposed on the membrane surface, show a high degree of respective complementary charge distribution. In the case of B870α there is a net positive charge, while the B870β N-terminus has a negative charge [5]. These data suggest that B870α and B870β interact on the membrane surface by charge combination. However, no cross-linking between the α and β chains has been observed [14]. In the primary structure a strong homology has been observed between B870α of *Rps. capsulata* and B870α of *Rps. sphaeroides* [15] as well as between B870β polypeptides of these bacteria [5,15]. The B870 antenna complex of *Rps. capsulata* is an oligomeric structure of the basic subunit (B870α, B870β, Bchl, carotenoid = 1:1:2:1) [2,3,14].

ACKNOWLEDGEMENTS

This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Eidgenössische Technische Hochschule Zürich.

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